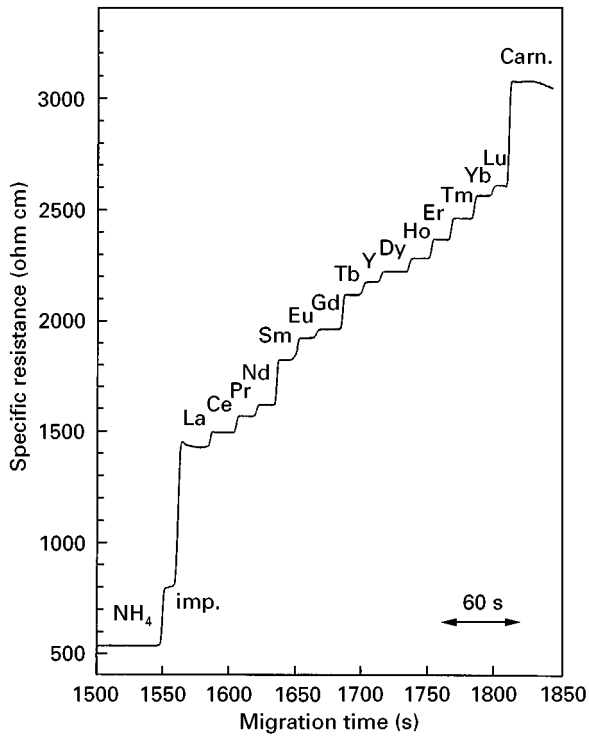

Figure 8 The observed isotachopherogram of 15 rare-earth ions (lanthanide ions and yttrium ion). HIBA, the complex-forming agent α-hydroxybutyric acid. The leading ion, 20 mmol L^{-1} NH_4^+; pH buffer = 2-ethyl-*n*-butyric acid ($pH_L = 4.8$). The sample amount was 0.33 mmol $L^{-1} \times 5$ μL. Migration current = 40 μA. The terminator is carnitine hydrochloride. (Carn.) imp., impurity of the used electrolyte system.

100 μL) can be injected. For preparative purpose, ITP is sometimes better than CE especially when the sample size is relatively large. In order to utilize the favourable features of ITP, an automated apparatus is needed or a method should be found to use commercial CE apparatus for ITP.

Further Reading

Babskii VG, Zhukov MYu and Yudovich VI (1989) *Mathematical Theory of Electrophoresis*. New York: Plenum.

Bocek P, Deml M, Gebauer P *et al.* (1988) In: Radola BJ (ed.) *Analytical Isotachophoresis*. Basel: VCH.

Everaerts FM, Beckers JL, Verheggen ThPEM (1976) *Isotachophoresis. Theory, Instrumentation and Application*. Amsterdam: Elsevier.

Gebauer P, Caslavska J and Thormann W (1991) *Journal of Biochemical and Biophysical Methods* 23: 97.

Hirokawa T, Nishino M, Aoki N *et al.* (1983) Table of isotachophoretic indicies. I. Simulated qualitative and quantitative indicies of 287 anionic substances in the range pH 3–10. *Journal of Chromatography* 271: D1–D106.

Li SFY (1992) *Capillary Electrophoresis, Principles, Practice and Applications*. Amsterdam: Elsevier.

Moscher RA, Saville AD and Thormann W (1992) *The Dynamics of Electrophoresis*. Weinheim: VCH.

Pospichal J, Gebauer P and Bocek P (1989) Measurement of mobilities and dissociation constants by capillary isotachophoresis. *Chemical Reviews* 89: 419–430.

DropBooks

Isotachophoresis in Capillary Electrophoresis

See **II / ELECTROPHORESIS / Capillary Isotachophoresis**

Mass Spectrometry Detection in Capillary Electrophoresis

See **II / ELECTROPHORESIS / Capillary Electrophoresis-Mass Spectrometry**

Micellar Electrokinetic Chromatography

M.-L. Riekkola, Laboratory of Analytical Chemistry, University of Helsinki, Finland

Copyright © 2000 Academic Press

Introduction

Micellar electrokinetic capillary chromatography (MEKC), first introduced by Shigeru Terabe and coworkers in 1984, has extended the potential of

capillary electromigration techniques to the separation of uncharged analytes. With its impressive separation efficiency and flexibility, MEKC has become a popular technique especially in the pharmaceutical and biomedical fields.

Above their critical micelle concentration (CMC), surfactant monomers added to an electrolyte solution form aggregates called micelles. Individual micelles are not significantly larger than the solutes being separated. On account of their small size and large number, they have a high surface area-to-volume ratio. Their structures are dynamic, with the average residence time of a surfactant monomer in the micelle being in the order of 1 ms or less. Separation in MEKC is based on the partitioning of analytes between the micelles and the aqueous phase, in the presence of electroosmotic flow. The micelles act as a pseudo-stationary phase. The mechanism of the analyte–micelle interaction is mainly determined by hydrophobic and electrostatic interactions. MEKC was originally developed to exploit the advantages of capillary electrophoretic techniques (high efficiencies, the requirement of only minute amounts of sample and reagent, fast analysis time) in the separation of neutral solutes of closely similar structure, but it is also applicable to the separation of charged compounds. A basic capillary electrophoresis (CE) instrument is used, and the separations are carried out usually in uncoated fused silica capillaries after hydrodynamic injection.

Separation in MEKC

When one or more micelle-forming surfactants are added to the electrolyte solution at concentrations above their CMC, partition of the analytes into the micellar pseudo-stationary phase increases the selectivity of the separation system. The overall separation of compounds is based on their differential solubilization into the micelles and on the migration velocities of the micelles under the electric field, in the presence of electroosmotic flow (EOF). The separation principle for an anionic surfactant is illustrated in **Figure 1**.

The separation of neutral analytes is based on their partitioning between the aqueous phase and the micellar stationary phase. When solutes interact strongly with the micelles their migration time is comparable to that of the micelles, t_{mc}, allowing the solutes to serve as micelle markers. Neutral analytes migrate with times t_1 and t_2, which lie inside a window formed by the migration times of the neutral

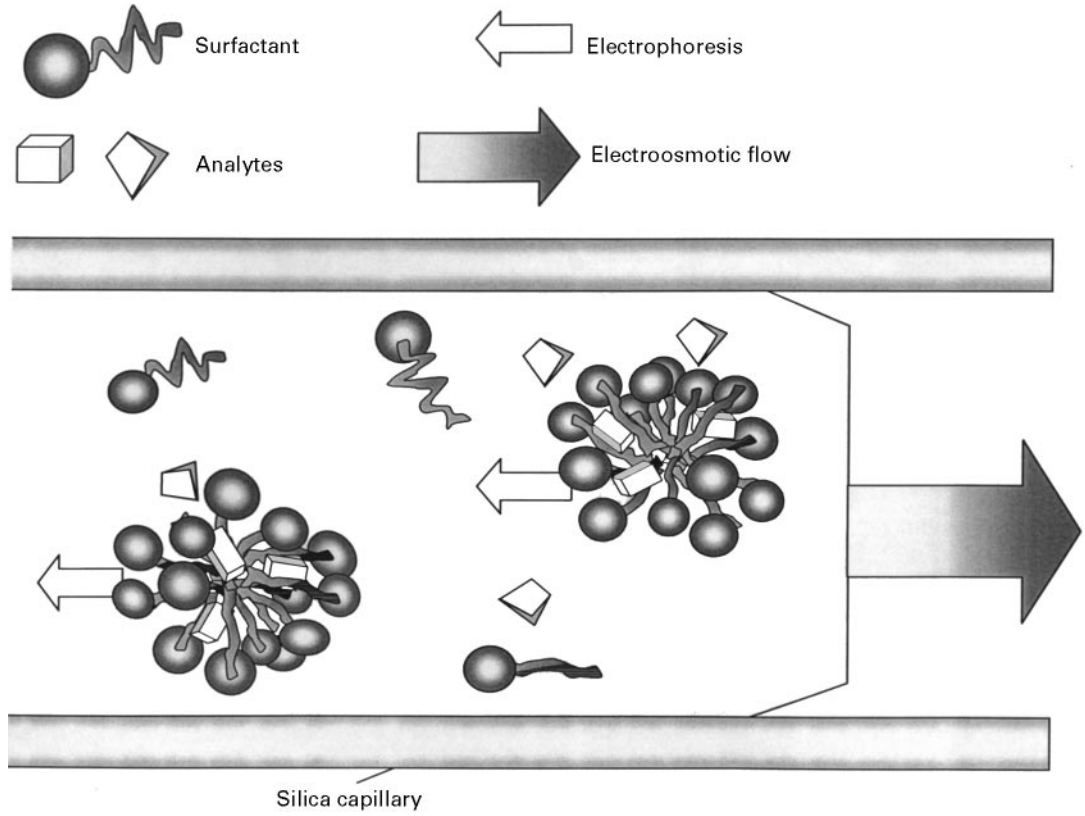

Figure 1 Schematic depiction of separation in micellar electrokinetic capillary chromatography.

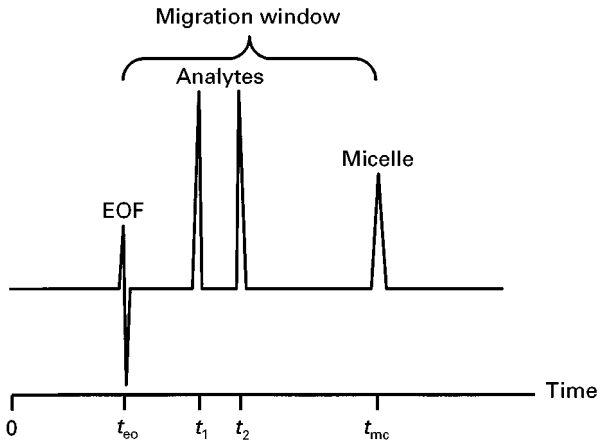

Figure 2 Migration window for neutral solutes in micellar electrokinetic capillary chromatography. EOF, electroosmotic flow.

electoosmotic flow marker, t_{eo}, and the micelle marker, t_{mc} (**Figure 2**). A relatively polar molecule (e.g., acetone, acetonitrile, formamide, methanol, 1-propanol or tetrahydrofuran) can be used as electroosmotic flow marker, and usually a highly hydrophobic, neutral compound such as Sudan III, Sudan IV, dodecanophenone, Orange OT, or Yellow OB as micelle marker. The migration window is finite because the micelles themselves migrate out of the capillary. Even though the peak capacity is restricted by the migration window, high separation efficiencies can be achieved. A wide migration time window is favourable for high resolution, but then a long analysis time may be required.

The micellar phase is not a true stationary phase because it is moving along the capillary towards the detector. When the analyte is permanently retained, its migration time (t_m) is identical with the migration time of the micelle (t_{mc}). Therefore, the term 'retention factor' used in chromatography should be replaced by the term 'partition factor' in MEKC. The partition factor k_{mekc} is described as:

$$k_{mekc} = \frac{n_{mc}}{n_{aq}}$$

where n_{mc} and n_{aq} are the numbers of the analytes in micellar and aqueous phases, respectively. In the case of a neutral analyte, k_{mekc} can also be calculated directly from the migration times:

$$k_{mekc} = \frac{t_m - t_{eo}}{t_{eo}(1 - t_m/t_{mc})}$$

However, there may be variations in k_{mekc} depending on EOF and the micelle marker; in particular, the choice of the micelle marker may have a significant effect on the value.

The selectivity α can then easily be determined by the ratio of the partition factors of two compounds:

$$\alpha = \frac{k_{mekc2}}{k_{mekc1}}$$

The most effective way to alter the selectivity of nonpolar analytes in MEKC is to change the micellar phase by changing the type of surfactant. When compounds are neutral, factors such as concentration of electrolyte and micellar solutions, pH, voltage and temperature have a relatively minor effect on the selectivity of the system. When the compounds are charged, on the other hand, variations in pH may induce changes in the dissociation of the compounds, affecting their charge, and thereby the solute–micelle ionic interactions and electrophoretic mobilities.

The resolution in MEKC is determined by the equation given by Terabe *et al.*:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_{mekc2}}{1 + k_{mekc2}}\right)\left(\frac{1 - t_{eo}/t_{mc}}{1 + (t_{eo}/t_{mc})k_{mekc1}}\right)$$

where N is the plate number. The resolution of the system depends on the efficiency, the selectivity, the partition factor and the migration time window.

DropBooks

Surfactants

Unique selectivities are achieved in MEKC through appropriate choice of anionic, cationic, nonionic and zwitterionic surfactants (**Table 1**). Surfactants are molecules with distinct hydrophobic and hydrophilic parts. The CMC increases dramatically with the alkyl chain length of the surfactant. At Kraft temperature, T_{Kr}, the solubility of the surfactant increases rapidly. T_{Kr} is the point at which surfactant solubility equals the CMC. The Kraft point varies with the surfactant, increasing with the length of the alkyl chain. Surfactant concentrations above the CMC and temperature above the Kraft point are required for the formation of micelles. Changes in temperature, concentration of surfactant, pH, ionic strength, additives in the aqueous phase and structural groups in the surfactant may cause changes in the size, shape and aggregation number of the micelles. In aqueous media, surfactants with bulky or loosely packed hydrophilic groups and long, thin hydrophobic groups tend to form spherical micelles, while those with short, bulky hydrophobic groups and small, close-packed hydrophilic groups tend to form lamellar cylindrical micelles. Factors that decrease the electrostatic repulsion between the head groups of ionic surfactants favour micelle

Table 1 Typical surfactants used in MEKC, with their critical micelle concentration (CMC)

Surfactant	*CMC (mM)*	*Temperature (°C)*
Anionic		
Sodium dodecyl sulfate (SDS)	8.2	25
Sodium tetradecyl sulfate (STS)	2.1	25
Sodium decyl sulfate	33	40
Sodium dodecyl sulfonate	11.4	40
Sodium *N*-lauroylmethyl-*N*-taurate	8.7	25
Lithium perfluorooctane sulfonate (LiPFOS)	6.3	25
Cationic		
Cetyltrimethylammonium bromide (CTAB)	0.92	25
Cetyltrimethylammonium chloride (CTAC)	1.3	30
Tetradecyltrimethylammonium bromide (TTAB)	3.6	25
Dodecyltrimethylammonium bromide (DTAB)	16	25
Dodecyltrimethylammonium chloride (DTAC)	20	25
Cationic fluorosurfactant (Fluorad FC 134)	na	
Nonionic and zwitterionic		
Octyl glucoside (OGLU)	25	25
Polyoxyethylene (23) dodecanol (Brij-35)	0.1	na
Polyoxyethylene (20) sorbitane monooleate (Tween 80)	0.01	na
Polyoxyethylene (20) sorbitane monolaurate (Tween 20)	0.059	na
3-[3-(Chloroamidopropyl) dimethylammonio]-1-propane-sulfonate (CHAPS)	4.2–6.3	na
Chiral surfactants		
Sodium *N*-dodecanoyl-L-valinate (SDVal)	2	na
Sodium *N*-dodecanoyl-L-glutamate (SDGlu)	na	
Digitonin (DIG)	na	
Bile salt surfactants		
Sodium cholate (SC)	12.5	25
Sodium deoxycholate (SDC)	10	25
Sodium taurocholate (STC)	4	25
Sodium taurodeoxycholate (STDC)	6	na
Sodium glycodeoxycholate	na	

na, not available.

formation leading to lower CMC in electrolyte solutions than in pure water.

Many physical properties change dramatically at the CMC. These changes can be exploited by determining the CMC of surfactants in CE electrolyte solutions, for example by measuring surface tension, light scattering, refractive index, electrical conductivity or electrophoretic mobility. The data are plotted against surfactant concentration, and a change in the slope corresponds to the CMC. However, the CMC obtained may differ according to the method used because micellization is a gradual aggregate growth which occurs over a finite concentration range. CMC values for the most commonly used surfactant, sodium dodecyl sulfate, in selected electrolyte solutions are listed in **Table 2**.

Anionic Surfactants

Anionic surfactant systems are preferred in MEKC because the electrophoretic migration of the micelles is in the opposite direction to the electroosmotic flow, and the micelles do not interact with the negatively charged walls of the fused silica capillaries. Anionic surfactants with alkyl chain and polar group, such as sodium decyl sulfate, sodium *N*-lauroyl-*N*-methyl-taurate, sodium tetradecyl sulfate, and especially sodium dodecyl sulfate (SDS) are the most widely used. Simultaneous separation of neutral and positively charged compounds is not possible at low pH because the EOF is too slow to carry the micelles to the cathode.

Most studies with anionic surfactants have been carried out under neutral or basic conditions. The most frequently used anionic surfactant, SDS, forms relatively spherical micelles with hydrophobic tail groups oriented towards the centre and charged head groups along the outer surface. The surfaces of SDS micelles possess a large net negative charge, giving them a large electrophoretic mobility toward the anode.

Another group of anionic surfactants, which has been widely used in separations of both neutral and ionic analytes, is bile salts. Bile salts have a hydroxyl-substituted steroidal backbone with hydrophilic and

Table 2 CMC values of SDS in selected electrolyte solutions at 25°C

Electrolyte solution	*CMC (mM)*	*Method of determination*
50 mM AMPSO[a] (pH 9.0)	3.6	Conductometric titration
50 mM AMPSO[a] (pH 9.0)	3.9	CE
50 mM AMPSO[a] (pH 8.7)	2.7	Surface tension
20 mM PIPES[b], 20 mM NaOH (pH 7.0)	3.8	Conductometric titration
100 mM BES[c], 100 mM NaOH (pH 7.0)	3.1	Conductometric titration
100 mM borate, 50 mM phosphate (pH 7.0)	2.9	Conductometric titration
5 M urea, 100 mM borate, 50 mM phosphate (pH 7.0)	4.4	Conductometric titration
20% DMSO (v/v), 25 mM sodium tetraborate, 50 mM sodium dihydrogen phosphate (pH 7.0)	6	Conductometric titration
20% acetone (v/v), 25 mM sodium tetraborate, 50 mM sodium dihydrogen phosphate (pH 7.0)	6.3	Conductometric titration
20 mM sodium tetraborate (pH 9.2)	3.1	CE
20 mM sodium tetraborate (pH 8.0)	5.5–9.6	CE
5 mM sodium tetraborate–acetonitrile (85 : 15, v/v)	7.3	CE
5 mM sodium tetraborate (pH 9.2)	5.3	CE
100 mM sodium tetraborate, 100 mM sodium dihydrogen phosphate (pH 6.0)	2	CE
100 mM sodium tetraborate, 100 mM sodium dihydrogen phosphate (pH 6.5)	2.4	CE
100 mM sodium tetraborate, 100 mM sodium dihydrogen phosphate (pH 7.0)	3.1	CE
100 mM sodium tetraborate, 100 mM sodium dihydrogen phosphate (pH 7.7)	4	CE
50 mM CHES[d] (pH 10.0)	2.9–5.2	CE
50 mM CHES[d] (pH 10.0)	2.7–5.4	CE
80 mM CHES[d] (pH 10.0)	1.6–2.2	CE
100 mM CHES[d] (pH 10.0)	1.2–2.4	CE
50 mM ammonium acetate (pH 9.0)	1.7–2.7	CE

[a]AMPSO = 3-[(1,2-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid; [b]PIPES = piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) monosodium salt; [c]BES = *N*,*N*′-bis(2-hydroxyethyl)-2-aminoethanesulfonic; [d]CHES = 2-(*N*-cyclohexylamino)ethanesulfonic acid.

DropBooks

hydrophobic faces and they form helical micelles. Bile salts have a lower solubilizing effect on hydrophobic compounds than does SDS.

Cationic Surfactants

Unlike anionic surfactants, positively charged surfactants, monomers and micelles are strongly attracted to the negatively charged surface of the fused-silica capillary wall and thus have a significant effect on EOF. Cationic surfactants such as long-chain alkylammonium salts may even cause a reversal of EOF through electrostatic interactions with the capillary surface, and this may occur at surfactant concentrations below the CMC. The capability for reversed EOF has been successfully exploited in MEKC separations.

Neutral and Zwitterionic Surfactants

Although neutral surfactants with zero electrophoretic mobilities cannot be exploited in the MEKC separation of nonionic solutes, they can be applied to the separation of ionic solutes. Since problems with Joule heat do not arise when nonionic surfactants are used at high concentration, large voltages can be used even when surfactants are added to the buffer in high concentration. Like the neutral surfactants, the zwitterionic surfactants do not contribute to the net conductivity of the electrolyte solution.

Mixed Micelles

Selectivity in MEKC can often be improved by using mixed surfactants. Clearly different selectivities from those obtained with the corresponding single micelles can be achieved, Some mixed micellar systems are presented in **Table 3**.

High Molecular Mass Surfactants

The high molecular mass surfactants used in MEKC are either oligomers of monomeric surfactants or block copolymers with surface-active properties. It has been proposed that the micelle is formed of a single molecule, and accordingly it has been termed a 'molecular micelle'. Because their CMC values are close to zero, molecular micelles are considered to be highly stable irrespective of the experimental conditions.

Surfactants and Cyclodextrins

Cyclodextrins (CD) are the most popular chiral selectors for chiral separations by MEKC. The separation mechanism is based on differential partitioning of solutes between the micellar and CD aqueous phase.

Table 3 Selected mixed micellar systems used in MEKC

Mixed micellar system	*Surfactants in the mixture*[a]
Anionic–nonionic surfactants	SDS and Brij-35
	SDS and Tween 60
	SDBS abd Brij-35
	SDS and Tween 20
	Bile salts and polyoxyethylene-4-dodecyl ether
Anionic–anionic surfactants	SDS and sodium cholate
	SDS and sodium octyl sulfate
	SDS and bile salts
	Two different bile salts
	LiPFOS (fluorocarbon) and LiDS (hydrocarbon)
Anionic–cationic surfactants	Fluorosurfactants FC 128 and FC 134
Anionic–zwitterionic surfactants	SDS and SB-12
Nonionic–nonionic surfactants	Tween 20 and Tween 80
	Triton X-100 and Brij-35
Cationic–cationic surfactants	TTAC and OTAC
	TTAB and DTAB

[a]SDS = sodium dodecylsulfate; Brij-35 = polyoxyethylene (23) dodecanol; Tween 20 = polyoxyethylene (20) sorbitane monolaurate; Tween 60 = polyoxyethylene (20) sorbitane monostearate; SDBS = sodium dodecyl benzenesulfonate; LiPFOS : lithium perfluorooctane sulfonate; LiDS = lithium dodecyl sulfate; SB-12 = *N*-dodecyl-*N*,*N*′-dimethyl-3-ammonio-1-propanesulfonate; TTAC = tetradecyltrimethylammonium chloride; OTAC = octyltrimethylammonium chloride; DTAB = dodecyltrimethylammonium bromide.

Most of the surfactants used in separations have been anionic.

Optimization of Separation

Resolution in MEKC is a highly complex and nonlinear function of experimental variables and is very difficult to optimize systematically. In a search for the optimal conditions for separation, several mathematical models have accordingly been developed. Often just a few test runs are needed to predict the best overall running conditions, though this naturally depends on the number of parameters included in the optimization strategy. When more than one surfactant is added to the electrolyte solution, the situation is complicated by the possible micelle–micelle interactions. Examples of the statistical optimization schemes used in MEKC are listed in **Table 4**.

Detection

Of the various detection systems employed in MEKC separations, optical systems are the most extensively used, and ultraviolet detectors (UV) used in conjunction with commercial CE instruments are a typical solution.

The sensitivity of mass spectrometry (MS), and the possibility of obtaining molecular information on compounds, make the on-line coupling of MEKC with MS highly attractive. Electrospray ionization (ESI) has been one of the most popular ionization techniques in coupled CE–MS. Although MEKC is a convenient separation technique for neutral analytes, problems are encountered in the on-line MEKC–ESI–MS interface connection because the micelles in the electrolyte solution are nonvolatile and tend to contaminate the MS. A number of approaches have been developed to overcome the problems of separating neutral compounds, while at the same time preventing micelles from entering the mass spectrometer. These include use of the heart-cut technique, high molecular mass surfactants, a semipermeable membrane interface, anodically migrating micelles, and the partial filling technique. An electrospray–chemical ionization interface is a possibility for certain types of online MEKC–MS applications.

Applications

MEKC has been applied to a wide variety of compounds, including phenols and chlorinated phenols, amino acids, several pharmaceuticals and their metabolites, porphyrins, peptides, nucleic acids, nucleosides and oligonucleotides. The capability for direct injection of biological fluids (plasma, serum, urine) is a special feature of electrokinetic capillary analysis. Effective solubilization of the biological matrix components by surfactants, and increased selectivities due to hydrophobic interactions with the micellar pseudo-stationary phase are evidently advantageous in bioanalysis. The use of MEKC for therapeutic and diagnostic drug monitoring has also proven to be of considerable value.

Future Directions

The great advantage of MEKC is the feasibility to manipulate the selectivity simply by changing the composition of the micellar phase. Even though several surfactants have shown their potential to act as micellar pseudo-stationary phase, the versatility of the technique and the range of applications can be further extended by developing new synthetic micelle-forming surfactants like polyelectrolytes or exploiting mixed micelles or biomembranes as pseudo-stationary phases. Understanding the mechanisms involved will greatly facilitate the systematic optimization of the large number of experimental parameters leading to better, faster, easier, and more reliable separations. In addition, studies are still needed to clarify new possibilities to couple MEKC with mass spectrometry.

Table 4 Statistical optimization schemes used in MEKC

Optimized parameter	*Parameters varied*	*Modelling*
Selectivity and resolution	pH, [SDS], [borate]	CCD[c], desirability functions
Selectivity and resolution	pH, [SDS], [sodium cholate], [AMPSO][a]	CCD, desirability functions
Resolution	[acetonitrile], [urea]	Iterative regression strategy
Resolution	9 for a stepwise screening, followed by 3: pH, [SDS], [acetonitrile]	Fractional factorial design, full factorial design, RSM[d]
Yield for the derivatization of some dipeptides	Reaction time, T, ionic strength, pH, [isopropanol]	Fractional factorial design, CCD, RSM
Selectivity	pH, [SDS]	Iterative regression strategy
Resolution	[SDS], [acetonitrile]	CABRO II[e]
Precision and efficiency	[SDS], V, T	FUMI[f]
Resolution	T, V, ionic strength, [SDS], [HPMC][b], [β-cyclodextrin]	PLS[g]
Resolution	[SDS], [urea]	CABRO II
Resolution	pH, [SDS]	CAMOS[h]
Resolution	pH, [buffer], [SDS], [SDS + sodium heptyl sulfate], [acetonitrile]	Plackett–Burman statistical design
Resolution	[SDS], [*N,N*-dimethylformamide], ionic strength	ORM[i]
Resolution	pH, [SDS], [tetrabutylammonium salt]	ORM
Resolution	pH, [SDS]	ORM
Resolution	[SDS], [isopropanol], [β-cyclodextrin]	Full factorial design
Resolution	pH, [SDS]	Full factorial design

[a]AMPSO = 3-[(1,2-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonicacid; [b]HPMC = hydroxypropyl methylcellulose; [c]central composite design; [d]response surface modelling; [e]computer-assisted bivariate resolution optimization II; [f]function of mutual information; [g]partial least squares; [h]computer-assisted multivate optimization strategies; [i]overlapping resolution mapping.
V, voltage; T, temperature.

DropBooks

Further Reading

Camilleri P (1998) *Capillary Electrophoresis, Theory and Practice*, 2nd edn, pp. 135–182. New York: CRC Press.

Guzman NA (1993) *Capillary Electrophoresis Technology*, pp. 65–87, 693–704. New York: Marcel Dekker.

Khaledi MG (1998) *High-Performance Capillary Electrophoresis*, pp. 77–140. New York: John Wiley.

Poole CF and Poole SK (1997) Interphase model for retention and selectivity in micellar electrokinetic chromatography. *Journal of Chromatography A* 792: 89–104.

Riekkola M-L, Wiedmer SK, Valkó IE and Sirén H (1997) Selectivity in capillary electrophoresis in the presence of micelles, chiral selectors and non-aqueous media. *Journal of Chromatography A* 792: 13–35.

Rosen MJ (1989) *Surfactants and Interfacial Phenomena*, 2nd edn, pp. 108–206. New York: John Wiley.

Terabe S, Otsuka K, Ichikawa K, Tsuchiya A and Ando T (1984) Electrokinetic separations with micellar solutions and open-tubular capillaries. *Analytical Chemistry* 56: 111–113.

Terabe S (1989) Electrokinetic chromatography: an interface between electrophoresis and chromatography. *Trends in Analytical Chemistry* 8: 129–134.

Vindevogel J and Sandra P (1992) *Introduction to Micellar Electrokinetic Chromatography*, pp. 1–231. Heidelberg: Hüthig Buch Verlag.

Microtechnology

T. McCreedy, University of Hull, Hull, UK

Copyright © 2000 Academic Press

Introduction

Electrophoresis is an established separation technique, frequently used for mixtures ranging from proteins and DNA to small anions and cations. However, perhaps its greatest strength lies in its remarkable ability to separate charged macromolecules. Reports describing electrophoretic separations started to appear in the 1930s, but the most significant developments really took place in the 1940s and 50s when separations with a paper or gel support matrix were used for the separation of macromolecules. The early